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How Should Long-Term Tunneled Central Venous Catheters Be Managed in Microbiology Laboratories in Order To Provide an Accurate Diagnosis of Colonization?

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Guidelines recommend the roll-plate technique for short-term central venous catheter (CVC) tip cultures. However, the issue of whether the roll-plate technique is better than the sonication method for long-term CVCs remains unresolved. In addition, no data are available for predicting the value of direct Gram staining in anticipating catheter colonization or catheter-related bloodstream infection (CRBSI) in these long-term CVCs. Our objectives were to compare the roll-plate technique and the sonication method and to define the validity values of Gram staining for the prediction of colonization and CRBSI in patients with long-term tunneled CVCs. During the study period, all tunneled CVCs removed at our institution were prospectively and routinely sent to the microbiology laboratory for Gram staining (first) and tip culture (the Maki technique and sonication, in a random order). We received 149 tunneled CVCs, 39 (26.2%) of which were colonized and 11 (7.4%) of which were associated with CRBSI. Overall, the roll-plate method detected 94.9% of the colonized catheters, whereas sonication detected only 43.6% ($P < 0.001$). The validity values of Gram staining for the detection of colonization and CRBSI were as follows: a sensitivity of 35.9% to 60.0%, a specificity of 100% to 94.2%, a positive predictive value of 100% to 42.9%, and a negative predictive value of 81.5% to 97.0%. The roll-plate technique proved to be better than sonication for the detection of bacteria in long-term tunneled CVCs. Gram staining of the tips of tunneled CVCs can anticipate a positive culture and rule out CRBSI. In our opinion, direct Gram staining should be incorporated into routine microbiological assessments of long-term catheter tips.

The recently reported Infectious Diseases Society of America (IDSA) *Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection* recommend the roll-plate technique for the routine clinical microbiological analysis of short-term catheter tips (9). However, for the analysis of long-term catheter tips, in which the endoluminal progression of microorganisms is more frequent, the best methodology for culture remains an unresolved issue (7, 12, 15).

Although the sonication of catheter tips can better detect the microbiological colonization of the inner surface, both the inner and outer surfaces can be colonized simultaneously (6). The results of the few studies comparing the roll-plate and sonication methods are not homogenous for catheter type or the sequence order of the procedure (2, 6, 11, 16). In the present study, it was demonstrated that the roll-plate technique was at least as efficient as sonication for the overall group of catheters received in the microbiology laboratory, although the number of long-term catheters was limited. Moreover, both techniques were compared in selected subpopulations with tunneled central venous catheters (CVCs).

Acridine orange and Gram staining have been used to provide a rapid diagnosis of catheter-related bloodstream infection (CRBSI) with and without the removal of CVCs with a short indwelling time (1, 5, 14); however, no information about this procedure is available for long-term CVCs.

The objectives of our study were to compare the roll-plate and sonication methods to diagnose catheter colonization and CRBSI and to assess the validity of Gram staining as a rapid procedure to predict or exclude colonization and CRBSI in patients with long-term tunneled CVCs.

MATERIALS AND METHODS

Setting. Ours was a prospective study performed between July 2009 and April 2011 at a large tertiary institution in Madrid, Spain.

We included all tunneled CVCs (Hickman and PermCath) that were routinely removed at the Vascular Interventional Radiology Department, irrespective of the reason for withdrawal. No antimicrobial-coated catheters were used during the study period.

Laboratory procedures. When the catheters arrived at the microbiology laboratory, the tips underwent Gram staining (first) and culture (roll-plate and sonication in a 1:1 random order).

Gram staining involved the rolling of the external surface of the distal catheter segment 3 times on a sterile glass slide with 2 to 3 drops (about 15 μl each) of sterile water on the surface. The slides were heat fixed and Gram stained by use of standard methods. The reading was taken at a magnification of $\times$100 along 3 longitudinal lines over 5 to 7 min, and the presence of at least 1 microorganism on the reading surface was considered a positive result. All the slides were read before the culture results were available.

A semiquantitative roll-plate technique described previously by Maki was performed by transferring each catheter tip onto a plate with Columbia agar supplemented with 5% sheep blood and rolling the tip back and forth across the surface at least 3 to 4 times (8).

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TABLE 1 Patient and catheter characteristics

Characteristic	Value
Median age of patients (yr) (IQR)	58.2 (16–87)
No. (%) of male patients	73 (52.9)
No. (%) of patients with underlying disease	
Cancer	80 (58.0)
Renal failure	58 (42.0)
Cumulative no. of catheter days	25,462
Median no. of catheter days (IQR)	137 (59–237)
Median no. of times of catheter use	40 (20–72)
No. (%) of patients with site of catheter insertion of:	
Jugular vein	133 (89.3)
Subclavian vein	16 (10.7)
No. (%) of patients with reason for catheter withdrawal of:	
End of use	85 (57.0)
Suspicion of bloodstream infection	40 (26.8)
Suspicion of local infection	15 (10.1)
Obstruction-malfunction	7 (4.7)
Thrombosis	2 (1.3)
No. (%) of patients with insertion site that was:	
Intact	124 (83.2)
Swollen	24 (16.1)
Ulcerated	1 (0.7)

Sonication was performed by placing the catheter tip into 10 ml of brain heart infusion broth, sonicating for 1 min (at 55,000 Hz and 125 W), and vortexing for 15 s. Again, 0.1 ml of the sonicated broth and 0.1 ml of a 1:100 dilution of the broth were streaked onto sheep blood agar plates (17).

The plates were incubated aerobically for 48 h at 37°C, followed by anaerobic incubation for 5 days at 37°C. The number of colonies recovered was counted.

For the analysis of the validity of Gram staining, the gold standard was defined as a positive catheter tip culture performed by using either the roll-plate technique or sonication.

Definitions. (i) Catheter colonization. Catheter colonization was defined as a positive semiquantitative tip culture by either the roll-plate technique (Maki technique) (≥15 CFU/plate) or sonication (≥100 CFU/plate) (8, 17).

(ii) CRBSI. CRBSI was defined as the isolation of the same microorganism(s) in both the catheter tip and at least 1 peripheral blood culture (9).

(iii) Possible or clinically suspected CRBSI. Possible or clinically suspected CRBSI was defined as the presence of clinical signs of catheter-related infection without the isolation of the microorganism in the catheter tip culture.

(iv) Exit-site infection. Exit-site infection (ESI) was defined as erythema, induration, and/or tenderness within 2 cm of the catheter exit site; this may be associated with other signs and symptoms of infection, such as fever or purulent drainage from the exit site, with or without concomitant bloodstream infection.

(v) Tunnel infection. Tunnel infection was defined as tenderness, erythema, and/or induration >2 cm from the catheter exit site, along the subcutaneous tract of a tunneled catheter, with or without concomitant bloodstream infection.

TABLE 2 Microorganisms isolated in colonized tunneled CVCs

Microorganism[a]	No. (%) of catheters with organism
Gram positive	34 (70.8)
Staphylococcus epidermidis	17 (35.4)
Staphylococcus aureus	6 (12.5)
CoNS	4 (8.3)
Propionibacterium acnes	4 (8.3)
Corynebacterium spp.	2 (4.2)
Clostridium bifermentans	1 (2.1)
Gram negative	10 (20.8)
Enterobacter cloacae	2 (4.2)
Stenotrophomonas maltophilia	2 (4.2)
Serratia marcescens	2 (4.2)
Klebsiella pneumoniae	1 (2.1)
Proteus mirabilis	1 (2.1)
Pseudomonas aeruginosa	1 (2.1)
Escherichia coli	1 (2.1)
Yeasts	4 (8.3)
Candida parapsilosis	2 (4.2)
Candida glabrata	1 (2.1)
Candida tropicalis	1 (2.1)
Total	48

[a] CoNS, coagulase-negative staphylococci.

RESULTS

We included 149 tunneled CVCs (89 Hickman and 60 PermCath CVCs) from 138 patients. The median indwelling time was 137 days (interquartile range [IQR], 59 to 237 days). The main underlying disease was cancer (58.0%), followed by renal failure (42.0%). Most catheters were removed due to an end of use (57.0%), followed by suspicion of or proven bloodstream infection (26.8%). Patient and catheter characteristics are detailed in Table 1.

The overall catheter tip colonization rate was 26.2% (39/149 CVCs), and the distribution of the isolated microorganisms was as follows: 70.8% Gram-positive organisms, 20.8% Gram-negative organisms, and 8.3% yeasts (Table 2). The colonization rate in those catheters that were removed because of an end of use was 24.7% (21/85 CVCs). The microorganisms isolated were as follows: 74.1% Gram-positive organisms, 14.8% Gram-negative organisms, and 11.1% yeasts. The roll-plate technique and sonication were performed first with 72 and 77 catheters, respectively. When the roll-plate technique was performed before sonication, catheter colonization rates were 36.1% and 16.7% for the roll-plate technique and sonication, respectively ($P < 0.001$); when the roll-plate technique was performed after sonication, colonization rates were 14.3% and 6.5%, respectively ($P < 0.001$). Similar results were obtained when the analysis was performed with different subpopulations according to clinical data (Fig. 1).

During the total of 25,462 cumulative catheter days, we found 11 episodes of definite CRBSI (incidence density of 0.43 episodes/1,000 catheter days). Details of the episodes are shown in Table 3. However, we found 40 patients with possible CRBSI and 25 patients with ESI (Table 1).

The validity values of Gram staining for the prediction of positive CVC cultures and CRBSI were as follows: a sensitivity of

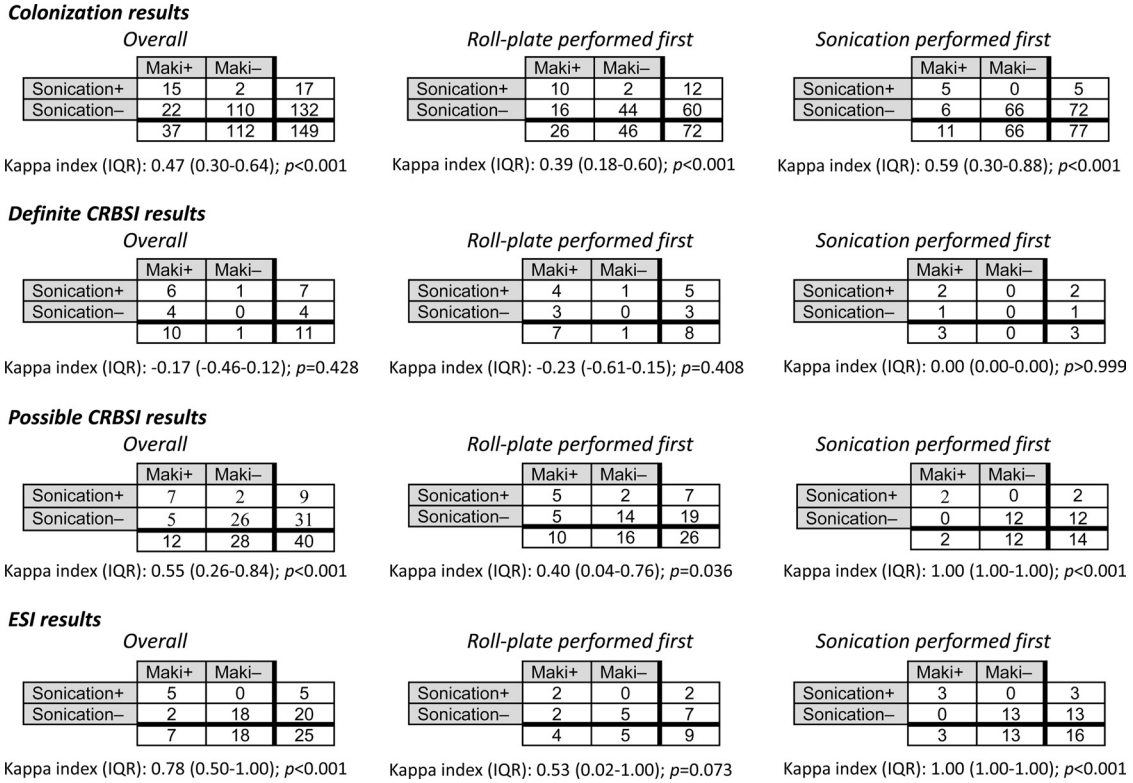

FIG 1 Comparison of the roll-plate technique and the sonication method.

35.9% to 54.5%, a specificity of 100% to 94.2%, a positive predictive value of 100% to 42.9%, and a negative predictive value of 81.5% to 96.3% (Table 4). However, the negative predictive values were better when the analysis examined CRBSI episodes caused by Gram-negative bacilli and yeasts (96.5% and 98.0%, respectively). Gram staining also had a high specificity when it was performed for the subpopulations of patients with possible CRBSI and with ESI (95.4% and 91.9%, respectively).

DISCUSSION

The roll-plate technique proved to be significantly better than sonication for the detection of positive CVC cultures. A negative Gram stain of the tunneled CVC tip has a high negative predictive value for colonization and CRBSI (definite and possible), particularly for Gram-negative bacteria and fungi.

Despite recommendations to perform the roll-plate technique for the diagnosis of colonization of short-term CVCs by the IDSA *Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection* (9), the issue of how to manage catheter tips from long-term tunneled CVCs remains unresolved in the most recent guidelines. Sherertz et al. (17) first described sonication as a quantitative procedure for the detection of catheter tip colonization, which proved to be suitable for distinguishing between infection and contamination (11). However, in their prospective study comparing the semiquantitative technique of Maki and the quantitative methods of sonication and vortexing for the detection of colonization of CVC tips and CRBSI, Bouza et al. (2) demonstrated no superiority of the quantitative techniques over Maki's technique. However, most of the 1,000 catheters included in that study had a short indwelling time.

Based on the fact that CVCs with a long indwelling time are more susceptible to colonization on the inner surface (7, 12, 15), sonication has been considered to be a better approach for the detection of colonization in this subgroup of CVCs. However, Kristinsson et al. (6) previously compared the roll-plate and ultrasonication methods for 236 CVC tips and demonstrated that even though culture from inside the catheters was the best predictor of infection, almost all infected catheters were colonized both on the inside and on the outside. Moreover, Slobbe et al. (18) performed a prospective and randomized study and demonstrated that sonication was no better than the roll-plate method for the detection of tip colonization or CRBSI in patients with long-term tunneled catheters. However, those authors included only patients with hematological conditions, and some CRBSI episodes were not confirmed, as in some cases, the only microbiological evidence was the differential time to positivity. Therefore, our study provides further evidence that the best methodology for the management of catheter tips from all patients with long-term tunneled CVCs is Maki's roll-plate technique.

Despite the low rate (8%) of subsequent bloodstream infections in patients with colonized tunneled catheters (10), the severe consequences and high mortality rates associated with this infection (3, 4, 13, 19) mean that anticipatory antibiotic therapy is necessary. This issue has been resolved for short-term CVCs using acridine orange and Gram staining, irrespective of whether the catheter was removed. Kite et al. (5) previously reported good sensitivity and specificity values (96% and 92%, respectively) for Gram staining and the acridine orange leukocyte cytospin (AOLC) test to detect CRBSI. Rushforth et al. (14) previously evaluated three tests for the diagnosis of infection in infants with

TABLE 3 Description of the 11 CRBSI episodes[a]

Characteristic[a]	Value
Median age of patients (yr) (IQR)	59.8 (42.5–68.6)
No. (%) of male patients	4 (36.4)
No. (%) of patients with underlying disease of:	
Cancer	5 (45.5)
Renal failure	6 (54.5)
Median comorbidity Charlson index (IQR)	4 (2–5)
Mean McCabe-Jackson index (SD)	2.82 (0.603)
Cumulative no. of catheter days	1,535
Median no. of catheter days (IQR)	119.5 (37.8–246.3)
No. (%) of patients with site of catheter insertion of:	
Jugular vein	11 (100)
Subclavian vein	0 (0.0)
No. (%) of patients with blood cultures drawn:	
Before catheter withdrawal	10 (90.9)
At the time of catheter withdrawal	1 (9.1)
After catheter withdrawal	0 (0.0)
No. (%) of patients with reason for catheter withdrawal of:	
Suspicion of bloodstream infection	10 (90.9)
Suspicion of local infection	1 (9.1)
No. (%) of patients with insertion site that was:	
Intact	7 (63.6)
Swollen	4 (36.4)
Median no. of DDDs (IQR)	11 (4.80–50.00)
Median total no. of days of therapy (IQR)	16 (6–27)
No. (%) of patients with antibiotic use before catheter withdrawal	9 (81.8)
No. (%) of patients with microorganism causing CRBSI	
Gram positive	7 (63.6)
Staphylococcus epidermidis	4 (36.4)
Staphylococcus aureus	3 (27.3)
Gram negative	3 (27.3)
Stenotrophomonas maltophilia	2 (18.2)
Proteus mirabilis	1 (9.1)
Yeasts	1 (9.1)
Candida tropicalis	1 (9.1)
Total	11

[a] DDDs, defined daily doses.

TABLE 4 Validity of Gram staining for detection of colonization and infection[a]

	% value			
Diagnosis	S	SP	PPV	NPV
Colonization	35.9	100	100	81.5
Definite CRBSI	60.0	94.2	42.9	97.0
Possible CRBSI	18.2	95.7	71.4	66.7
ESI	16.0	91.9	28.6	84.4

[a] S, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

suspected catheter sepsis and found that the AOLC test was 87% sensitive and 94% specific. Bouza et al. (1) demonstrated previously that catheter tip staining before culture was an easy-to-perform and effective procedure to anticipate catheter colonization and rule out CRBSI in short-term CVCs. However, none of those studies evaluated the validity of Gram staining for long-term tunneled CVCs. Therefore, our results show that this instant and easy procedure performed on tunneled catheters was 100% specific for tip colonization and had a 97.0% negative predictive value for the presence of definite CRBSI.

The main limitation of this study was the low number of definite CRBSI episodes and the fact that some CRBSI episodes could have gone undetected, as we did not obtain routine blood cultures from all the patients included. However, an analysis of different subpopulations based on clinical data was performed in order to recover possible CRBSI episodes which were not microbiologically confirmed.

In conclusion, we demonstrated that the best option for the detection of colonization of long-term tunneled CVCs was Maki's roll-plate technique performed on the catheter tip. We also found that Gram staining performed before culture had both high specificity and negative predictive values for the prediction of catheter colonization and CRBSI, respectively.

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We declare no conflicts of interest.

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